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NEWSLETTER

8th Anniversary of the Fe*Bart*® Test©

Evelyn E. Zuckerman, Editor **Fall 2007** **Vol. 6, Number 4**

In This Issue:

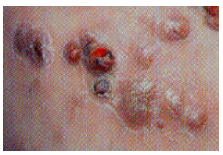
November 5, 2007 was the 8th anniversary of our *Bartonella* testing service. We have performed 159,196 Fe*Bart*® screening tests and 9,792 therapy titration tests from 3,073 hospitals in the USA, Canada, and the Caribbean since November 5, 1999. **We thank all the veterinarians and veterinary technicians who have helped us gather biological evidence of the importance of *Bartonella* in cats, dogs and people.** In the Fall 2007 Newsletter, Dr. Hardy will reflect on our work with *Bartonella* and relate it to our previous retrovirus research.

Reflections

William D. Hardy, Jr., V.M.D.
Director

In The Beginning:

Cat Scratch Disease (CSD) was first described by Parinaud, a French physician, in 1889.[1] The etiology of CSD remained a mystery for more than a century, until in 1990 Relman and his colleagues, using recombinant DNA technology discovered the bacterial cause from an HIV-infected person.[2] At that time, we were investigating the human retroviruses, HIV-1, HIV-2, HTLV-I and HTLV-II at the Memorial Sloan Kettering Cancer Center.[3] Later, at the Center for Infectious Diseases, Bronx Lebanon Hospital Center, Albert Einstein College of Medicine, Dr. Hardy learned that the clinicians were treating some seriously ill HIV-infected patients who were infected with *Bartonella*.



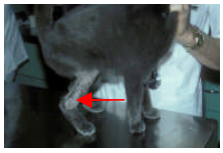
HIV infected patient with bacillary angiomatosis of the skin caused by *Bartonella henselae* transmitted from his kitten.

Epidemiological studies found that the "cat scratch disease" agent- *Bartonella* was transmitted from cats to people and, as a veterinarian working in a human hospital research setting, it seemed a great opportunity to employ our previous FeLV retrovirus test expertise toward the development of an accurate and practical test for detection of *Bartonella* in cats. We were interested in determining the prevalence of *Bartonella* infection in pet cats and whether or not the bacteria caused diseases in cats similar to those being found in people.

After 5 years of development we found that the most accurate, sensitive and reproducible test for detection of *Bartonella* antibodies was the western immunoblot (WB).[4] The WB has the advantage of detecting the full range of all the antibodies produced against the *Bartonella* proteins by cats. This prevents false positive tests due to cross-reactive antibodies to other microorganisms. Thus, on November 5, 1999 we performed our first *Bartonella* test for the veterinary profession. That first test was submitted from a healthy 4-year-old male DSH by Dr. Jan Rottenberg, Just Cats Veterinary Care, Edison, NJ.

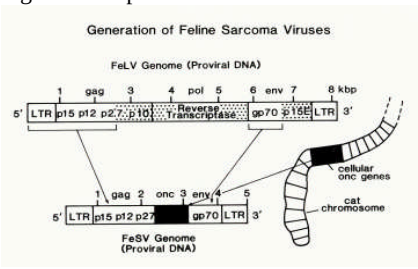
Previous Research Relationship:

Previous to our interest in *Bartonella*, we spent 24 years in my laboratory, the Laboratory of Veterinary Oncology at Memorial Sloan Kettering Cancer Center, NYC, studying cancer in cats and dogs and retroviruses of cats, humans, mice and cattle. We were the first to show that L-asparaginase was an effective cancer chemotherapeutic agent, that AZT was effective in stopping the replication of a retrovirus- FeLV (later to be the first anti-HIV agent approved) and the first to show that any retrovirus was transmitted contagiously in any animal (FeLV horizontal transmission among cats).[5,6] In 1986 Evelyn Zuckerman, in my laboratory, isolated a feline sarcoma virus (HZ-4 FeSV) from a pet cat which contained a unique viral oncogene.[7]

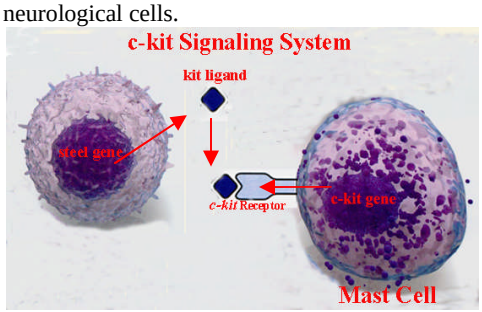


A young cat with multicentric fibrosarcoma of the hock containing a FeSV with the *v-kit* oncogene.

A viral oncogene is a normal cellular gene that is transduced (taken from) and integrated into the FeLV genome to produce a sarcoma virus.



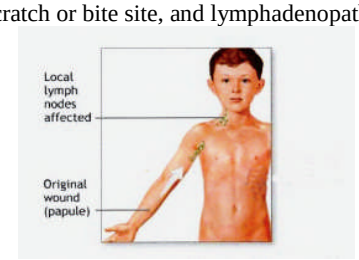
When the newly formed (*de novo*) FeSV infects a susceptible cat cell it can transform it into a cancer cell (sarcoma cell). Our colleague Dr. Peter Besmer in my group, characterized the viral oncogene and named it *v-kit*.[7] The analogous normal cellular gene is called *c-kit* and the gene encodes a cellular receptor called the *c-kit* receptor. The ligand (factor) that reacts with the *c-kit* cell receptor is called the *kit* ligand. *C-kit* is expressed on certain tissues such as gut cells, mast cells, reproductive cells and some neurological cells.



Mutations in the *c-kit* gene have been associated with tumors of the GI tract, testes, mast cell tumors and other tumor types.

Bartonella Immunopathology:

The basic response to *Bartonella* infection in all animals is chronic inflammation characterized by lymphocytic, plasmacytic infiltrates, granuloma formation, and lymphadenopathy. The prodrome (earliest consistent signs) of CSD consists of 1 or all of the following signs: fever, skin papule at the scratch or bite site, and lymphadenopathy.

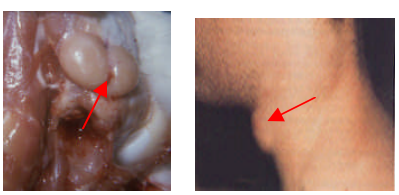


CSD prodrome:
Fever, skin papule and lymphadenopathy

Lymphadenopathy occurred in 71 of the 84 cases of human CSD that we studied.[8] An 8 year summary of our findings of lymphadenopathy in cats and dogs is presented in the Table below.

Lymphadenopathy and *Bartonella*

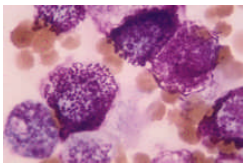
Species:	# *Bartonella* +/ Lymphadenopathy	%
Cat	1,653/3,641	45%
Dog	40/287	14%



Feline and human lymphadenopathy caused by *Bartonella henselae*.

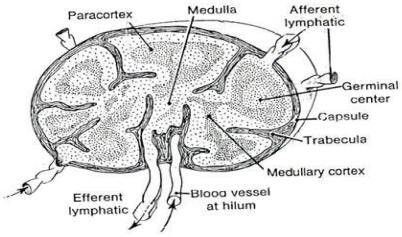
C-kit and *Bartonella*:

Our work with *Bartonella* is related to *c-kit* by the correlation of *c-kit* with mast cells and mast cells to inflammation. Mast cells express *kit* receptors and are very important in both normal and abnormal immune responses such as allergy IBD, and autoimmunity.



Feline mast cells with dark-staining granules containing numerous mediators.

Recent research has elucidated the mechanism that causes lymph nodes to swell or enlarge as a response to infection.[9] The reaction of lymph node swelling is called lymphadenopathy and the process is found to be orchestrated by mast cells. Lymphadenopathy is a common result of *Bartonella* infection in cats, dogs and people. In fact, the most common feature of cat scratch disease is a regional lymphadenopathy of a lymph node or lymph nodes that drain the site of the scratch or bite that transmitted the bacteria.

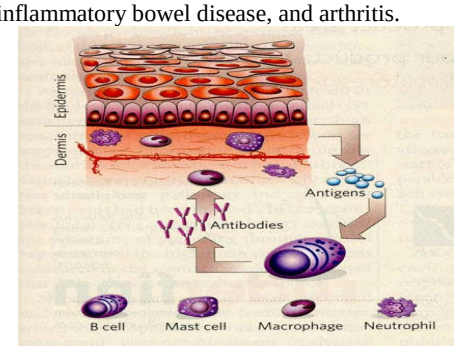


Anatomy of a normal lymph node

Lymph nodes that drain the site of *Bartonella* entry are the center of the adaptive immune response and entrap large numbers of circulating lymphocytes. Here newly recruited naïve T lymphocytes interact with and are sensitized by *Bartonella* antigen loaded antigen presenting cells (dendritic cells from the periphery) and begin the adaptive immune response of reactive T cells and B-cells. Until recently, the signal that causes lymph nodes to begin to react to the distal infection was unknown. That signal has been found to be the release of tumor necrosis factor (TNF) from mast cells positioned as guardians in peripheral tissues, exposed to the external environment, such as the skin, mucous membranes and the GI tract.

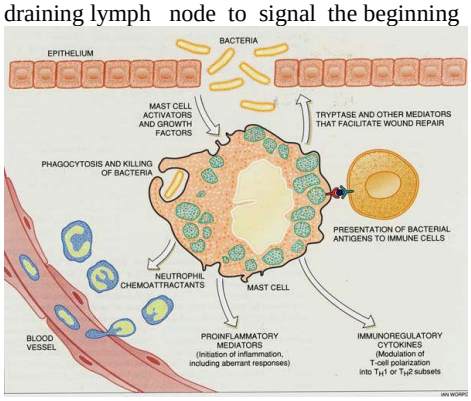
TNF was originally identified in mouse serum after injection with *Mycobacterium bovis* strain bacillus Calmette-Guerin (BCG) and endotoxin. Serum from such animals produced hemorrhagic necrosis, and in some instances, complete regression, of certain transplanted tumors in mice.[10] As the name specifies, TNF causes necrosis in the tumors.

Nearly all cells display receptors for TNF on their surfaces. Their responses to TNF, however, can be very different although TNF signaling is usually used for defense against infection. TNF can direct an infected cell to destroy itself by apoptosis, and the presence of lipopolysaccharide on bacterial surfaces stimulates blood cells to release TNF, which promotes an inflammatory response to fight the infection. Because TNF plays such diverse and often contradictory roles, the body must keep a careful balance to ensure that TNF is applied only when and where it is needed. When this control is lost, it can lead to severe inflammatory illnesses such as septic shock, inflammatory bowel disease, and arthritis.



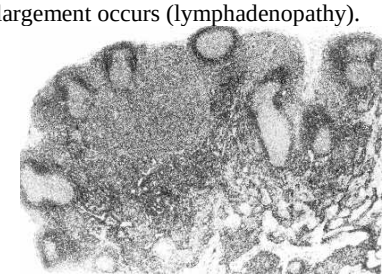
Mast cells and other immune cells poised in the skin and under mucous membranes to sense invasion by microorganisms.

Many cells can produce TNF which is an important mediator for the immune system but only mast cells produce and store TNF. The stored TNF can be released within minutes of mast cells recognizing an invading bacterium such as *Bartonella.* The TNF travels to the draining lymph node to signal the beginning



Mast cells detect *Bartonella* invasions and release their preformed soluble TNF which travels to draining lymph nodes to begin the preparation of an immune defense.

of immune reactivity. Immune activated lymph nodes show a crowding in of lymphocytes from the periphery and loss of their normal architecture (hypertrophy) and enlargement occurs (lymphadenopathy).



Reactive lymph node- hypertrophy

Thus the release of the soluble preformed TNF from mast cells, stationed in the skin, in recognition of *Bartonella*, travels to the draining lymph nodes and is responsible for the lymphadenopathy seen in infected cats and people. Mast cells do not need to migrate to the lymph nodes and exert their effect remotely.

More references are available at:
www.nlm.gov or www.scholar.google.com

These basic immune system observations were made with genetically modified mice using the *c-kit* gene to manipulate mast cell numbers. We are proud that our discovery of *v-kit* from a cat fibrosarcoma enabled the observations of the mechanisms of the immune system to be made.

Postscript:

As luck would have it, in 1975 as a young Post Doctoral Fellow in Dr. Lloyd Old's Laboratory of Tumor Immunology in the Memorial-Sloan Kettering Cancer Center in New York City, I witnessed, and was asked to photograph, the experimental animals in which TNF was discovered.[10]

Discovery of Tumor Necrosis Factor (TNF) in 1975 from a mouse with a necrotic tumor.

At the time of this writing the following number of references on PubMed at the National Library of Medicine:
87,557 TNF, 1,743 FeLV & FeSV, 5,742 c-kit & v-kit, and 2,838 *Bartonella* & Cat Scratch Disease.

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